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Adaptation to High Light Intensity in *Synechococcus* sp. Strain PCC 7942: Regulation of Three *psbA* Genes and Two Forms of the D1 Protein

RESHAM D. KULKARNI AND SUSAN S. GOLDEN*

Department of Biology, Texas A&M University, College Station, Texas 77843-3258

Received 17 September 1993/Accepted 7 December 1993

The three *psbA* genes in the cyanobacterium *Synechococcus* sp. strain PCC 7942 encode two distinct forms of the D1 protein of photosystem II. The *psbAI* message, which encodes form I, dominates the *psbA* transcript pool at low to moderate light intensities; however, exposure to high light triggers a response in which the *psbAI* message is actively degraded while *psbAII* and *psbAIII*, which encode form II, are transcriptionally induced. We addressed whether these changes result from a generalized stress response and examined the consequence of light-responsive *psbA* regulation on the composition of D1 in thylakoid membranes. Heat shock and oxidative stress had some effect on levels of the three *psbA* transcripts but did not produce the responses generated by an increase in light intensity. Prolonged exposure to high light (24-h time course) was characterized by elevated levels of all *psbA* transcripts through maintenance of high levels of *psbAII* and *psbAIII* messages and a rebound of the *psbAI* transcript after its initial decline. Form II-encoding transcripts were enriched relative to those encoding form I at all high-light time points. Form II replaced form I in the thylakoid membrane at high light despite an abundance of *psbAI* transcript at later time points; this may be explained by the observed faster turnover of form I than form II in the membrane. We propose that form II is less susceptible to damage at high light and that this qualitative alteration, coupled with increased turnover of D1, protects the cells from photoinhibition.

Photosystem II (PSII) is a membrane-embedded protein-pigment complex involved in key reactions of oxygenic photosynthesis: charge separation in response to excitation of a chlorophyll molecule by light and by oxygen evolution. This mode of photosynthesis, which requires two photosystems (PSII and photosystem I), is conserved in cyanobacteria, algae, and higher plants. The reaction center of PSII contains two structurally similar proteins, D1 and D2, which harbor the photoreactants involved in the primary reactions. D1 and D2, along with cytochrome b_{559} and the 4.8-kDa *psbI* gene product, form the core of the PSII reaction center (16, 31, 33).

Light intensity modulates the composition of thylakoid membranes in plants as well as in cyanobacteria to best use the available light (2, 3, 30). This involves variation in the relative proportions of light-harvesting pigments, PSII and photosystem I reaction centers, electron carriers, and ATP synthase (2). On exposure to light intensities in excess of those found in optimal growth conditions, a decline in photosynthetic capacity, i.e., oxygen evolution and CO_2 fixation, which is termed photoinhibition, occurs. The D1 protein is believed to be the initial site of photoinhibition, with its damage reflected in the loss of PSII activity (26, 35). The D1 protein of plants is rapidly turned over in the light, with the rate being proportional to light intensity. Thus, it is thought to play a role in protection of PSII against photoinhibition and may be designed to be removed and replaced as fast as possible (35). Light-induced turnover of D1 has also been demonstrated in the cyanobacterium *Synechococcus* sp. strain PCC 7942 (15, 21, 36).

Expression of two different forms of D1, encoded by *psbA* multigene families, is a unique cyanobacterial characteristic not observed in higher plants. *Synechococcus* sp. strains PCC 7002 and PCC 7942 and *Synechocystis* sp. strains PCC 6714 and PCC 6803 each possess three *psbA* genes (5, 12, 14, 20), whereas *Anabaena* sp. strain PCC 7120 has four *psbA* genes (42). In the glucose-tolerant derivative of *Synechocystis* sp. strain PCC 6803 that is widely used for PSII structure-function analysis, the *psbA-2* and *psbA-3* genes are nearly identical, whereas *psbA-1* is more divergent (32). The *psbA-2* and *psbA-3* transcript levels in this strain are stimulated by light; the *psbA-1* gene is not expressed. In *Anabaena* sp. strain PCC 7120, *psbAII*, *psbAIII*, and *psbAIV* predict identical polypeptides that differ from the putative *psbAI* product by 21 amino acid residues. The *psbAI* gene and one or more of the other *psbA* genes are expressed in PCC 7120 under standard laboratory conditions (42). In higher plants, the *psbA* transcript is chloroplastic and increases in abundance during chloroplast biogenesis and in response to light (17).

The regulation of a *psbA* multigene family is best understood in *Synechococcus* sp. strain PCC 7942. The product of *psbAI*, form I of the D1 protein, differs by 25 amino acid residues from form II, which is encoded by *psbAII* and *psbAIII* (14). The three members of the gene family are differentially expressed in response to changes in light intensity (9, 37, 38). Each of the *psbA* genes gives rise to a monocistronic 1.2-kb message; an additional *psbAII* transcript (1.6 kb) initiates further upstream and carries a second open reading frame (9). The levels of the 1.2-kb *psbAII* and *psbAIII* messages increase rapidly when cells are shifted to high light intensity, whereas the *psbAI* transcript level decreases. Induction of both *psbAII* and *psbAIII* transcripts is attributable to an increase in transcription from these genes and not to increased transcript stability (25). The decline in the *psbAI* transcript level is caused by accelerated degradation of this message; the *psbAIII* message, but not that of *psbAII*, is also subject to this posttranscriptional regulation at high light. The 1.6-kb *psbAII* transcript is not regulated by light intensity (9).

* Corresponding author. Phone: (409) 845-9824. Fax: (409) 845-2891. Electronic mail address: sgolden@tamu.edu.

These responses of the PCC 7942 *psbA* genes occur during the first 30 min after a shift to high light. The aim of the present work was to monitor regulation of the *psbA* genes during adaptation to a high-light environment and to examine the consequences of differential *psbA* response for the D1 composition of the thylakoid membrane. Another objective was to test whether any of the *psbA* responses are in part due to high light intensities triggering a generalized stress response. We found that the high-light response is distinct from those elicited by heat shock or oxidative stress. The long-term response to high light was characterized by elevated levels of *psbA* messages, through maintenance of high levels of *psbAII* and *psbAIII* transcripts and a rebound of the *psbAI* transcript succeeding its initial decline. At high light intensity, form II replaced form I in the thylakoid membrane despite a high abundance of the *psbAI* transcript at later time points; this may be explained by the observed faster turnover of form I than form II in the membrane at high light intensity.

MATERIALS AND METHODS

Culture conditions. Wild-type *Synechococcus* sp. strain PCC 7942 was grown in BG-11 medium (1) as modified by Bustos and Golden (8). For all studies, cells were grown in a turbidostat (8) as a continuous culture in which cells were maintained at a constant cell density (optical density at 750 nm, 0.5) and a constant light intensity. Cultures were incubated at 30°C with aeration as well as efficient mixing of the culture achieved by bubbling with 1% CO_2 in air. The turbidostat culture served as the source of cells adapted to standard light intensity (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) for all experiments. Light intensity (photosynthetic photon flux density [PPFD]) at the center of the culture was measured by an immersible probe (Biospherical Instruments, San Diego, Calif.) and reported as microeinsteins per square meter per second.

For high-light studies, the PPFD was increased to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ by moving the light source towards the culture growing in the turbidostat. For studies in which either rifampin or paraquat was added to the culture, or for heat shock treatment, cells were collected from the turbidostat and transferred to Pyrex tubes. These tubes were suspended in an aquarium maintained at 30°C or at 45°C for heat shock studies, and the cultures were bubbled with 1% CO_2 in air. PPFD was varied as described for each experiment. Cells were harvested at the time points indicated, poured over crushed ice, pelleted immediately, and frozen in liquid nitrogen. All samples were stored at −85°C for RNA extraction or for thylakoid membrane isolation.

Stress treatments. Cells adapted to standard PPFD were subjected to the following stress conditions. To create heat shock stress, the temperature of the aquarium was increased to 45°C prior to the transfer of cells from the turbidostat. To create oxidative stress conditions, paraquat (Sigma Chemical Co., St. Louis, Mo.) was added to the cells to give a final concentration of 500 μM.

RNA methods. RNA was extracted from PCC 7942 cells as described previously (25). RNA samples (5 μg based on a reading at an optical density of 260 nm) were denatured with formaldehyde, separated by electrophoresis on a 1.2% agarose gel, and blotted onto a charged nylon membrane (Magnacharge; Micron Separations Inc., Westboro, Mass.) as described by Ausubel et al. (4). Blots were probed with radioactive antisense transcripts from plasmids that contain fragments from the unique upstream untranslated regions of each of the *psbA* genes (7). The antisense RNAs were produced by using an in vitro transcription kit and [α-^{32}P]UTP (Ambion Inc., Austin, Tex.). A *Cla*I-*Sal*I fragment from the *groEL* gene (43) was gel purified and radiolabeled by using a random-primer labeling kit and [α-^{32}P]dCTP (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Hybridization and washing conditions were the same as those described previously by Bustos et al. (9).

RNase protection assays were performed with the RPA kit (Ambion). Radiolabeled RNA probes were synthesized as described above except that unlabeled UTP at a final concentration of 5 μM was added to each reaction mixture. Incorporation of [α-^{32}P]UTP into RNA was determined by comparing counts per minute of trichloroacetic acid-precipitable material with that of an unprecipitated aliquot of the reaction mixture. The full-length radiolabeled RNA probes were identified on a 12% polyacrylamide gel and eluted from gel slices by incubation for 1 h at 37°C in buffer provided in the Ambion kit. PCC 7942 total RNA samples (0.5, 1.0, 1.5, 2.0, and 2.5 μg) were hybridized with radiolabeled probes. The different concentrations of RNA were used to ensure that the probe was in excess and the quantitation was linear. After overnight hybridization of RNA samples with each of the *psbA* probes, single-stranded regions of the hybrids were digested with RNases provided in the kit. Twice the amount of Dx solution recommended in the kit instructions was required to precipitate these RNA hybrids, which are shorter than the species used by Ambion to develop the protocol. Samples were separated on a 12% polyacrylamide gel, and protected fragments were cut out of the gel and subjected to scintillation counting. Values were normalized for the specific activities of the three probes on the basis of the number of uridine nucleotides in their sequences.

To calculate RNA half-lives in high-light-adapted PCC 7942, cells growing in the turbidostat at a standard PPFD were shifted to high PPFD. Six hours after the high-PPFD shift, cells were transferred to Pyrex tubes in the presence or absence of rifampin (Sigma; 200 μg/ml) and were subjected to continued high PPFD. RNA was extracted from samples collected at appropriate time points and used for Northern (RNA) blot analysis. To ensure even loading of total RNA in the lanes, the 16S RNA bands from photographic negatives of the ethidium bromide-stained gels were scanned with a Bio-Rad model 620 densitometer, and small corrections were made from these values. Autoradiograms of appropriate exposure were scanned, and half-lives were calculated from the equation generated by subjecting the data to exponential regression.

Isolation of thylakoid membranes and immunoblot analysis. Cells growing at standard PPFD in the turbidostat were shifted to high PPFD and harvested at several time points. Thylakoid membranes were isolated as described by Schaefer and Golden (38). Membrane fractions were stored at −85°C in small aliquots. Solubilized membranes were separated by lithium dodecyl sulfate-polyacrylamide gel electrophoresis (18), and the proteins were electroblotted onto nitrocellulose as recommended by Ausubel et al. (4). Immunoblot analysis was performed by using an ECL Western blotting (immunoblotting detection kit (Amersham International plc, Buckinghamshire, England). Blots were probed with antisera specific for form I or form II of D1 (38) or an anti-D1 antiserum raised against the protein from spinach (41) which recognizes both form I and form II of D1 in PCC 7942. Immunoblot conditions were those recommended by Amersham. The secondary antibody was horseradish peroxidase-conjugated anti-rabbit antibody. Blots were exposed to X-ray film after incubation with the detection reagents.

Turnover rates of the two forms of D1 were calculated from cells that were grown at standard PPFD and shifted to high PPFD for 30 min before the addition of chloramphenicol (250

μg/ml; Sigma) to block new protein synthesis. Cells were harvested at intervals starting 5 min after the addition of chloramphenicol for thylakoid membrane isolation and immunoblot analysis. Densitometry and calculation of protein half-lives were performed as those used for RNA half-lives.

Computer-generated images. An Apple OneScanner and Ofoto software (version 1.1; Light Source Computer Images, Inc.) were used to generate graphic images. The files were transferred to Canvas (version 3.0.6; Deneba Systems, Inc.) for lettering and printed on a Tektronix Phaser IISD dye-sublimation printer.

RESULTS

The *psbA* light response is distinct from known generalized stress responses. We wanted to know whether the *psbA* response to high light represents a distinct light-dependent signal pathway or is actually part of a generalized stress response triggered by the sudden change in light intensity. We tested whether any of the characteristic *psbA* light responses are seen in cells subjected to well-established stress conditions. In *Escherichia coli*, a shift from 30 to 45°C results in a dramatic increase in the production of heat shock proteins, among which are the GroEL and GroES proteins (19). The *groESL* operon has been sequenced from PCC 7942, and its message level increases dramatically when cells are shifted from 30 to 45°C (43). Our heat shock treatment reproduced this result (data not shown). The *psbAI* message level decreased slightly by 15 min after heat shock but returned to its initial level within 30 min. Transfer to high light also causes the *psbAI* message level to decrease, but it does not come back up within 30 min of exposure to high PPFD (9). The *psbAII* and *psbAIII* transcripts rapidly declined upon heat shock but showed some recovery within 60 min of exposure to high temperature. This is not similar to the characteristic light-induced increase of *psbAII* and *psbAIII* messages at high light (9, 25). A gradual increase in the *groESL* message level was observed in cells shifted to high PPFD, but it was not comparable in magnitude to the heat shock response (data not shown).

Oxygen radicals produced during electron transport reactions within the photosynthesis machinery have been postulated to damage the D1 protein (40). Since oxygen radicals may be formed when cells are shifted to high light, we followed the *psbA* response in cells subjected to oxidative stress. Paraquat, which is a redox cycling agent known to create oxidative stress conditions in vivo (11), was added to PCC 7942 cells. The *psbAII* and *psbAIII* message levels did not change rapidly but increased by about 3- and 10-fold, respectively, by 60 min after the addition of paraquat (data not shown). Even though we saw an eventual increase in *psbAII* and *psbAIII* message levels, the change did not correspond to the rapid transcriptional induction seen with the high-light response. The levels of these messages were actually lower 15 min after the addition of paraquat than before the addition. The *psbAI* message level decreased to 40% of the control value by 60 min after the addition of paraquat. The *groESL* message level was not enhanced under these conditions. Oxidative stress has not been well characterized in PCC 7942 with respect to gene regulation, and we did not have a probe for an oxidative stress-induced message to confirm generation of the stress.

All three *psbA* genes are expressed at a higher level in cells adapted to high light. We changed the intensity of illumination of a turbidostat (continuous) culture from standard PPFD to high PPFD and sampled cells over a time course to determine whether the rapid changes in *psbA* transcript levels are maintained in cells subjected to high light over longer periods. The

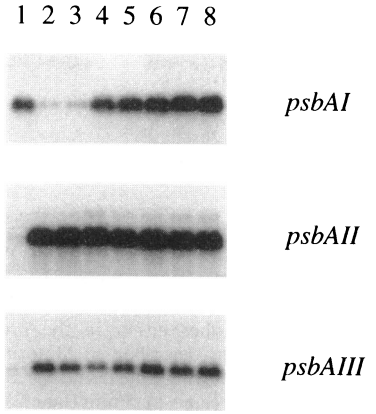

FIG. 1. Time course of *psbA* transcripts after transfer of cells from standard PPFD to high PPFD. Cells growing at standard PPFD (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) were shifted to high PPFD (500 $\mu E \cdot m^{-2} \cdot s^{-1}$), and samples were collected at the following time points after exposure to high PPFD for RNA isolation and Northern blot analysis: 0 h or standard PPFD (lane 1), 0.5 h (lane 2), 1 h (lane 3), 3 h (lane 4), 6 h (lane 5), 9 h (lane 6), 12 h (lane 7), and 24 h (lane 8). The blots were probed with antisense RNA probes specific for *psbAI*, *psbAII*, or *psbAIII* to detect the transcript indicated for each panel.

psbAII and *psbAIII* transcript levels increased and remained high throughout the 24 h of sampling (Fig. 1). The increases in the *psbAII* and *psbAIII* messages were about 20- and 8-fold, respectively, after 30 min at high light intensity, as reported previously (25). The *psbAI* transcript level decreased after the shift to high PPFD and remained low up to 1 h; however, the level then increased to about 1.5-fold its value at standard PPFD by 6 h after the light shift (Fig. 1). Thus, levels of all three *psbA* transcripts increase in cells adapted to higher PPFD, and the decrease in the *psbAI* transcript is only a transient response.

The increase in the *psbAI* transcript seen after the cells adapt to high PPFD (Fig. 1, lane 4) could be a result of either increase in transcription of *psbAI* or cessation of accelerated degradation of the message. We shifted cells to high light for 6 h before adding rifampin to measure the stability of the *psbA* messages after adaptation of the cells to high PPFD. Northern blot analysis showed that the *psbAIII* transcript is still degraded at the faster rate typically seen for cells subjected to high light (25) (Table 1; Fig. 2). However, the *psbAI* message half-life did not reflect the accelerated decay rate seen immediately after a shift to high light but was the same as that observed when rifampin was added before the light shift, blocking synthesis of the putative light-induced degradation factor (Table 1). Thus,

TABLE 1. Half-lives of the *psbA* messages at high light intensity

Transcript	Half-life (min) when rifampin was added:	
	At 5 min before shift to high light[a]	At 6 h after shift to high light[b]
psbAI	25.0 ± 3.6	24.7 ± 2.9
psbAII	29.5 ± 2.7	34.1 ± 3.2
psbAIII	11.6 ± 3.7	5.5 ± 0.5

[a] Half-life values for rifampin added 5 min before the light shift are from Kulkarni et al. (25).

[b] The experiment was done as described in the legend to Fig. 2. Data were obtained by densitometry of the autoradiograms, and half-lives were calculated by regression analysis. The values are the means of two experiments, and the range between these experiments is indicated.

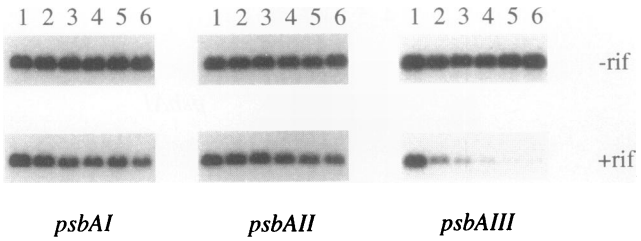

FIG. 2. Stability of the *psbA* messages at high PPFD. PCC 7942 cells growing at standard PPFD (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) were shifted to high PPFD (500 $\mu E \cdot m^{-2} \cdot s^{-1}$). Six hours after the light shift, cells were transferred to Pyrex tubes either in the presence (+rif) or absence (−rif) of rifampin as described in Materials and Methods. Cells were collected at the following time points for RNA isolation and Northern blot analysis: 0 min (lane 1), 5 min (lane 2), 10 min (lane 3), 15 min (lane 4), 20 min (lane 5), 25 min (lane 6). Blots were probed with antisense RNA probes specific for each of the *psbA* genes.

the *psbAI* message is restabilized after prolonged exposure to high PPFD, which accounts, at least in part, for the increase in the level of *psbAI* transcript after several hours at high PPFD.

Relative and absolute amounts of transcripts encoding form II of D1 increase at high PPFD. Previous experiments indicated that for every 100 *psbA* transcripts, 94 are from *psbAI*, 1.4 are from *psbAII*, and 4.6 are from *psbAIII* (14). These studies were not done under controlled light conditions, but the illumination was lower than the standard light intensity used in Fig. 1, lane 1. We performed RNase protection assays to determine whether the increase in *psbAII* and *psbAIII* transcript levels at high light significantly biases the *psbA* transcript pool to favor form II-encoding messages. The RNA samples were from (i) cells adapted to standard PPFD, (ii) cells subjected to high PPFD for 30 min, and (iii) cells subjected to high PPFD for 6 h. These time points correspond to lanes 1, 2, and 5 of Fig. 1 and represent the *psbA* population at standard light, at the maximum induction of form II-encoding transcripts, and after the rebound in *psbAI* transcript abundance. The results of the analysis are shown in Fig. 3. The *psbAI* transcript predominated at standard PPFD, constituting 82% of the total *psbA* message population. This ratio changed dramatically within 30 min after the shift to high light, with *psbAII* transcripts forming 80% of the *psbA* message population. The fraction of form II-encoding transcripts remained higher after cells were exposed to high light for 6 h than at standard light, i.e., 66% compared with 18%. Total *psbA* transcript levels also increased 300 to 400% by 6 h at high light intensity.

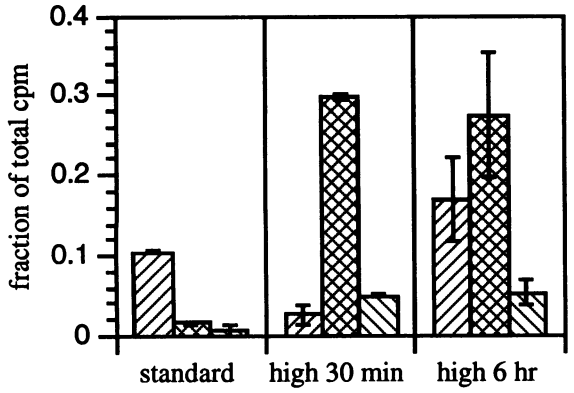

FIG. 3. Comparison of the abundance of the *psbAI* (▨), *psbAII* (▩), and *psbAIII* (▧) transcripts at standard PPFD (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) and after transfer to high PPFD (500 $\mu E \cdot m^{-2} \cdot s^{-1}$). Relative amounts of the transcripts were determined by an RNase protection assay using radiolabeled gene-specific probes as described in Materials and Methods. The value of each band (in counts per minute) is reported as a fraction of the total counts per minute of all samples at all time points, such that all bar heights can be compared. Bar heights indicate the means of values from two experiments, and the variation between experiments is shown by range bars.

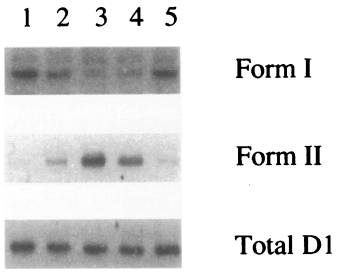

FIG. 4. Changes in the D1 composition of thylakoids at high PPFD. PCC 7942 cells were transferred from standard light (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) to high light (500 $\mu E \cdot m^{-2} \cdot s^{-1}$) and back to standard light. Thylakoid membranes were isolated from samples collected at standard PPFD (lane 1), at 0.5 and 6 h (lanes 2 and 3, respectively) after the shift to high PPFD and at 0.5 min and 6 h (lanes 4 and 5, respectively), after the shift down to standard PPFD. Thylakoid membranes were subjected to lithium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membranes which were then probed with the following antisera: anti-form I, anti-form II, and anti-D1. The anti-D1 serum was raised against D1 protein from spinach and it recognizes both form I and form II in PCC 7942.

Rapid light-dependent changes in composition of the D1 pool. We monitored the levels of form I and form II of D1 in thylakoid membranes with form-specific antisera (38) to see whether protein changes accompany the rapid changes in the *psbA* RNA population. Immunoblot analysis showed that the total D1 pool, as detected by anti-spinach D1 antiserum which recognizes both forms of D1, did not change significantly in response to a shift to high PPFD (Fig. 4). The total D1 protein level decreased slightly at high PPFD and increased again when cells were shifted back to standard PPFD (lanes 4 and 5). The quality of the D1 pool was, however, radically changed. The level of form II increased upon a shift to high light, whereas form I barely could be detected by 6 h after the shift (lane 3). Form II disappeared when cells were shifted back to standard light, whereas the level of form I recovered (lane 5). These results point to two conclusions: (i) the increase in the *psbA* message levels at high light does not result in an increase in the total D1 in the thylakoid but may compensate for the known high turnover of the protein at high PPFD; and (ii) even though *psbAI* transcript levels increase after adaptation to high PPFD (Fig. 3), no corresponding increase in the amount of form I in the thylakoid is apparent.

Turnover of the two forms of D1 at high light intensity. One possible explanation for the undetectable levels of form I after 6 h at high PPFD (Fig. 4, lane 3), in spite of abundant *psbAI* transcript (Fig. 3), is that there is a difference in the turnover of the two forms of the D1 protein. We calculated the turnover of form I and form II by monitoring their disappearance after blocking new protein synthesis with chloramphenicol. We selected a window of time during high-light adaptation when both forms of D1 could be detected easily. Cells growing at standard PPFD were shifted to high PPFD, and chloramphen-

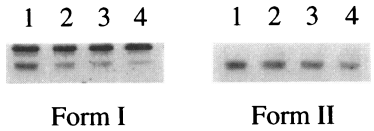

FIG. 5. Turnover of form I and form II of D1 at high PPFD. Cells growing at standard PPFD (130 $\mu E \cdot m^{-2} \cdot s^{-1}$) were transferred to high PPFD (500 $\mu E \cdot m^{-2} \cdot s^{-1}$), and chloramphenicol was added 30 min later. Cells were harvested at the following time points after chloramphenicol addition for thylakoid isolation and immunoblot analysis: 0 h (lane 1), 0.5 h (lane 2), 1 h (lane 3), and 1.5 h (lane 4). Blots were probed with anti-form I and anti-form II antisera. A strongly cross-reacting band, unrelated to D1, is evident in the anti-form I panel; its detection on immunoblots varies with the antiserum dilution.

icol was added to the culture 30 min after the light shift (equivalent to Fig. 4, lane 2). Cells were harvested at several time points for isolation of thylakoid membranes and immunoblot analysis. Figure 5 shows that form I turns over at a faster rate than form II at high light. The film images were scanned on a densitometer, and half-lives of the proteins were calculated by exponential regression of the data. Form I had a half-life of about 36 min, and form II had a half-life of about 90 min. These results indicate a difference between form I and form II which may play a role in efficient functioning of these two proteins under different light conditions.

DISCUSSION

The high-light responses of the *psbA* genes, namely, transcriptional induction of *psbAII* and *psbAIII* and an accelerated decay of *psbAI* and *psbAIII* transcripts (25), could be directly mediated by changes in light intensity or indirectly through other pathways. We addressed whether shifting cells from standard PPFD to high PPFD triggers a generalized stress response which accounts for the observed changes in gene expression. When PCC 7942 cells were heat shocked, levels of both *psbAII* and *psbAIII* transcripts decreased within 15 min (data not shown). In cells subjected to oxidative stress, the levels of *psbAII* and *psbAIII* transcripts were high at 60 min after the addition of paraquat, but at 15 min, the levels were even lower than those before the addition of the drug (data not shown). On the basis of these results, we conclude that the rapid transcriptional induction of *psbAII* and *psbAIII*, which can be observed even within 5 min of a high PPFD shift (25), is not a heat shock or oxidative stress response. All three *psbA* message levels were low 15 min after the heat shock and oxidative stress treatments. Thus, the stress response appears to be a transient decay of all three *psbA* messages. Light-induced degradation of *psbA* messages is more specific, since only *psbAI* and *psbAIII* messages turn over faster when cells are shifted to high light (25). We cannot rule out the possibility that turnover of *psbAI* and *psbAIII* at high light may be similar to a stress response. *psbAI* message levels decrease when cells are shifted to high PPFD or to high temperature, although the magnitude and the length of the two responses are different.

We observed that *groESL* message levels, which are highly induced (120-fold) upon heat shock (43), do not increase immediately when cells are shifted to high PPFD but do gradually increase to levels intermediate between standard PPFD and heat shock values. A gradual increase in *groESL* expression might be expected on the basis of an increase in metabolic activity of the cells at high light intensity and thus an increase in biosynthesis requiring molecular chaperones. We did not have a probe that could indicate whether shifting cells to high light increases transcripts from genes that are sensitive to oxidative stress.

The long-term high-light response of the *psbA* genes is the maintenance of high levels of *psbAII* and *psbAIII* transcripts and a rebound of the *psbAI* message by 3 h after the high PPFD shift (Fig. 1). Restabilization of the *psbAI* message after adaptation to high light contributes to its recovery (Table 1). We showed previously that both transcription and translation are required after a shift to high PPFD for the accelerated turnover of *psbAI* and *psbAIII* messages. When rifampin is added 10 min after the light shift, the half-lives of these messages are shorter than when rifampin is added before the light shift, showing that active degradation of these two messages occurs but requires a period at high light to synthesize a putative degradation factor (25). Figure 2 and Table 1 showed that, in cells adapted to high light, the *psbAI* message is restabilized; the *psbAIII* transcript, however, continues to be degraded at the faster rate, indicating a differential regulation of the *psbAI* and *psbAIII* transcript stabilities. We have not determined conclusively whether changes in *psbAI* promoter activity contribute to the observed increase in the *psbAI* message after adaptation to high light. A *psbAI-lacZ* reporter strain consistently shows a small decrease in β-galactosidase activity after exposure to high light (29), but a subsequent increase at later time points was not observed (28).

Brusslan and Haselkorn (7) reported an unexplained increase in the steady-state levels of *psbAII* transcript in a culture which had been passaged continuously over a period of several years, which they designated as WT_c. They proposed that the culture might have acquired a mutation which increased *psbAII* expression. However, subsequent analysis of the *psbAII* promoter in this strain showed no evidence of mutation (6), and we did not observe elevated *psbAII* levels in WT_c when they provided us with the strain (data not shown). Our current analyses suggest that Brusslan and Haselkorn observed elevated *psbAII* message levels in high-light-adapted wild-type cells. Their measurements were made before we had established the light-regulated nature of the three *psbA* genes and while they were attempting to achieve optimal growth conditions for RNA analysis in PCC 7942; this involved keeping cell cultures dilute, which improves light penetration (7). The quantitation of the *psbA* transcript pool composition in the WT_c strain by Brusslan and Haselkorn matches well our results for cells that have been at high PPFD for 6 h (7).

We often saw fluctuations in the levels of the *psbA* transcripts during long-term sampling as were seen for the *psbAIII* messages in Fig. 1. Figure 3 shows that there was significant variation between the two experiments in *psbA* message level quantitation at the 6-h time point after the light shift, but not at the 0- or 0.5-h time points. The variations were also apparent from Northern blot analysis of these samples. One cause of variation may be that the shift to high PPFD serves to trigger circadian rhythmicity of these genes. Expression of *psbAI* shows circadian cycling (22); circadian rhythms of the *psbAII* and *psbAIII* transcript levels also have been observed after cells were subjected to high light (27).

The *psbA* response in cells adapted to high light is an increase in all three of the *psbA* transcripts, with an overall three- to fourfold increase in the total *psbA* message (Fig. 1 and 3). The total D1 protein levels are not significantly altered; D1 abundance decreased slightly upon a shift to high light but recovered when cells were shifted back to standard light. Increased *psbA* gene expression may thus serve one main purpose, i.e., to increase D1 protein synthesis to compensate for the increased turnover of D1 at high PPFD. D1 is well known for its increased turnover at high PPFD (26, 35); this

has also been demonstrated in PCC 7942 (15, 21, 36). Expression from the *psbD* gene family in PCC 7942, in which two genes code for identical D2 polypeptides, increases in response to high light (8). This induction appears to compensate for faster turnover of D2 at high light.

The composition of the *psbA* transcript population changes at high light with *psbAII* and *psbAIII*, the form II-encoding messages, forming the predominant fraction. This is particularly evident immediately upon shift to high light, as represented by the 30-min sample (Fig. 3). Thus, the first few hours after the shift to high PPFD may be a critical transition period in which PCC 7942 cells increase synthesis of form II of D1 and actively promote its insertion into the membrane in place of form I. Our results showed that a rapid substitution of form II in place of form I occurs at high light. A concurrent study from another lab showed interchange of the two forms during photoinhibition (10). One of the important questions is whether form II is better suited for high-light growth and survival. Studies with mutants have shown that R2K1, a strain that has *psbAI* inactivated and thus produces just form II of D1, is more resistant to photoinhibition than the wild type or R2S2C3, which has both *psbAII* and *psbAIII* inactivated (10, 23). Thermoluminescence studies on mutants lacking form I or form II indicate that there may be a functional difference between these two forms (13). We have shown that there is a difference in the turnover of the two proteins at high PPFD (Fig. 5), i.e., 36 min for form I and 90 min for form II. Even though the *psbAI* transcript is abundant after several hours at high light, levels of form I are insignificant in the thylakoid membrane. The half-life of total D1 protein at high PPFD as detected by anti-D1 antiserum corresponds to the half-life of form II, which confirms the absence of a measurable quantity of form I in the thylakoid membranes (data not shown). In general, the turnover of D1 protein increases with increasing light intensities, which may serve to rid PSII of D1 protein that is damaged and needs to be replaced faster at high light (34, 35, 39). Whether form II, which has a longer half-life, is better than form I for high-PPFD growth is intriguing for this reason. R2S2C3, which lacks form II, has impaired growth upon shift to high PPFD compared with R2K1, which has only form II of D1 (24). Further experiments are directed towards establishing the specific functional differences of the two forms of D1 and the biological significance of regulating their synthesis differentially in relation to light intensity.

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